THE EFFECT OF AMMONIUM SALTS ON PROTOPLASM OF AMŒBA.

FLOYD J. BRINLEY,[1]

Physiological Laboratory, Battle Creek College, Michigan.

There has been a vast amount of work done on the effect of chemicals on plant and animal cells. The methods employed in studying this effect have been largely confined to immersion of the cells in solutions of the various reagents. It is well known that immersed cells may be affected in a number of ways: the reagent may act on the plasma membrane, may affect the internal protoplasm without injuring the membrane or it may affect both the membrane and the internal protoplasm. Until the advent of the micropipette (Barber, 1911; Kite, 1915; Chambers, 1922) it was not possible to ascertain how the substances affect or react with a cell. The work of Chambers (1926), Reznikoff (1926), Pollack (1927), Hiller (1927) and others have brought to light, by microinjection studies, many facts concerning the differences between the plasma membrane and the internal protoplasm and their reactions with various chemicals. Many substances such as narcotics, carbon dioxide, hydrogen cyanide, hydrogen sulfide, picric acid and certain salts which are lethal to immersed cells (amœbæ) have been found to be only reversibly injurious when injected into the cell. The action of strong acids and bases has been found (Chambers and Reznikoff, 1926) to be largely confined to the surface of the cell. HCl, pH 3, and NaOH, pH 9, when injected into amœba do not irreversibly injure the internal protoplasm, while amœbæ immersed in these solutions die very quickly.

From the work of Harvey (1911), Jacobs (1920) and others it appears that strong acids and bases enter living cells very slowly whereas weak acids and alkalies penetrate cells with little if any resistance. It has been rather generally accepted that the toxicity

[1] National Research Fellow 1927–1928. The author wishes to express his appreciation to Dr. C. E. McClung, University of Pennsylvania, for the use of a laboratory during the tenure of the fellowship.

of the weak acids and bases is due to their ease of penetration. However, the question of whether their toxicity is due to the effect on the plasma membrane or on the internal protoplasm has not been satisfactorily answered. Recently it has been shown that CO_2 (Chambers and Reznikoff, 1926), HCN and H_2S (Brinley, 1927 and 1928) when injected into amœba do not kill the cell unless the dosage is so large that it ruptures the plasma membrane. On the other hand, amœba die very quickly when immersed in these solutions. These experiments seem to prove conclusively that CO_2, HCN, and H_2S exert their lethal action primarily on the cell membrane and not on the internal protoplasm.

In view of the fact that ammonia enters cells very rapidly, in this respect being similar to the weak acids, it was thought desirable to ascertain whether the toxicity of ammonium hydroxide and other ammonium salts was due to their action on the plasma membrane or on the internal protoplasm. The present paper deals with the effect of certain ammonium salts, namely, hydroxide, chloride, citrate, phosphate and acetate on the protoplasm of amœba as determined by immersion and injection experiments.

Immersion Experiments.

Amœbæ were immersed in solutions of the following ammonium salts: hydrate, chloride, citrate, phosphate and acetate, and their effects on the organisms were studied. The species of amœba was not determined but it undoubtedly belonged to the proteous group. The concentrations of the solutions were N/10 and N/100. No attempt was made to control the H ion concentration but the pH was determined in each case by the colorimetric method. The reaction of the amœba to each salt will be discussed separately.

Ammonium hydroxide: Amœbæ immersed in N/10 ammonium hydroxide (pH 9.8) withdrew their pseudopodia and assumed a spherical form. The plasma membrane ruptured within a few seconds and the cell disintegrated. When amœbæ are immersed in N/100 solution the cell assumes a spherical form and swells slightly. Brownian movement becomes very rapid. The cell membrane dissolves within 3 to 5 minutes and the fluid protoplasm disperses into the surrounding solution.

Ammonium chloride: Amœbæ immersed in N/10 NH_4Cl, pH 6, elongate into the limax form and continue locomotion at a reduced rate for over an hour. The viscosity of the protoplasm seems to be slightly increased. At the end of two hours locomotion ceases, the animal rounds up and the cell disintegrates. When amœbæ are immersed in N/100 solution they assume the limax form and locomotion continues for over eighteen hours.

Ammonium carbonate: Immersion of amœbæ in N/10 or N/100 $(NH_4)_2CO_3$ results in an immediate cessation of locomotion, the pseudopodia remain extended and there appears to be a slowing down in the rate of Brownian movement. The cells swell slightly and the granules collect near the center of the cell and the protoplasm coagulates.

Ammonium acetate: Amœbæ placed in N/10 acetate solution continue to move at a slow rate for two hours. Finally the cell assumes a spherical form and disintegrates within two or three hours. They remain alive and continue locomotion in N/100 solution for over eighteen hours.

Ammonium citrate: Amœbæ immersed in N/10 or N/100 citrate solution elongate into the limax form and resume locomotion. The cell finally rounds up and the protoplasm coagulates.

Ammonium phosphate: Amœbæ placed in N/10 or N/100 phosphate solution ($NH_4H_2PO_4$) continue locomotion for several hours. The streaming of the protoplasm becomes sluggish and finally the protoplasm coagulates and the animal dies.

TABLE I.

THE COMPARATIVE TOXICITY OF CERTAIN AMMONIUM SALTS TO *Amœba proteus* (?).

Salt.	Concentration.	Time Required to Kill 75 per cent of the Organisms.	pH
Hydroxide	N/10	15 to 30 seconds	9.8
	N/100	3 to 5 minutes	
Carbonate	N/10	1 hour	8.5
	N/100	1.5 hours	
Citrate	N/10	5 to 10 minutes	5.4?
	N/100	3.5 hours	
Phosphate	N/10	2 hours	6.8
	N/100	4 hours	
Chloride	N/10	2 hours	5.0
	N/100	Alive after 18 hours	
Acetate	N/10	3 hours	6.4
	N/100	Alive after 18 hours	

The prominent feature of these experiments is the marked resistance of amœbæ to the ammonium salts. The salts, with the exception of the hydroxide, produce an increase in viscosity of the protoplasm and death is accompanied by coagulation of the protoplasm. The toxicity of the hydroxide may be due to the alkalinity of the solution. Table I. gives a summary of the comparative toxicity of the ammonium salts to amœbæ. The time of death is only approximately correct for it is very difficult to determine the exact death point.

Injection Experiments.

The ammonium salts used in the immersion experiments were injected into amœbæ by means of Chambers' micromanipulator. The concentrations used were N/1 to N/100. The salts, with the exception of the carbonate, were non-lethal even in high concentrations (N/1) when injected into amœba in amounts equal to one fourth the volume of the cell. Injections of normal solutions of the hydroxide, chloride, phosphate, acetate and citrate result in a local elevation of the membrane in the form of a blister near the point of entrance of the pipette. The solutions rapidly diffused throughout the cell, producing a reversible gelation of the protoplasm. The animals gradually withdrew their pseudopodia and assumed a spherical form. Eventually, streaming of the protoplasm occurs, Brownian movement is resumed and the organism recovers. Usually one large pseudopodium is formed and the animal adopts a limax form. The rate of recovery depends upon the salt injected. Cells injected with the chloride and phosphate require a much longer time for recovery than those injected with citrate, acetate and hydroxide. The approximate rate of recovery is as follows: chloride > phosphate > citrate > acetate > hydroxide.

A normal solution of ammonium carbonate is lethal to amœbæ when injected in amount equal to the volume of the nucleus. Injections of N/1 or N/10 solutions of the carbonate result in an initial increase in viscosity, the animal withdraws its pseudopodia and becomes spherical; finally the cell membrane dissolves and the protoplasm remains as a gelatinous mass. The cell recovers from a dosage of N/100 ammonium carbonate.

Tearing the Plasma Membrane.

Amœbæ were immersed in N/10 and N/100 solutions of the above ammonium salts and the cell membrane torn with microdissection needles. If a small tear is made in the membrane a portion of the internal protoplasm escapes but the cell rapidly forms a new membrane over the injured surface. The rapidity of the formation of the membrane depends upon the salt used. More protoplasm escapes from a tear in the membrane when the cells are placed in the hydrate and carbonate than in the chloride, acetate, citrate or phosphate.

Discussion.

Ammonium salts hydrolyze to different degrees depending upon the acid radical which is combined with the ammonia. The entrance of ammonia into the cell from solutions of ammonium chloride has been studied by Jacobs (1922), who has conclusively shown that a cell (Rhodendron or starfish egg) may develop an intracellular alkalinity when placed in a solution of ammonium chloride which is decidedly acid. This change in internal pH is undoubtedly, as Jacobs concludes, due to the selective permeability of the cell membrane. Chambers (1922) has verified this conclusion by injecting ammonium chloride into starfish eggs, thereby producing an intracellular acidity which demonstrates that the selective permeability is confined to the membrane and not to the internal protoplasm.

Harvey (1911) has shown by using intracellular neutral red as an indicator that ammonia and its primary, secondary and tertiary alkyl substitution products enter cells with very little if any resistance and that death of the cell does not result from the intracellular alkalinity as is evident by the ability of the cell to recover when removed from the ammonia solution and placed in pure water. On the other hand, Harvey concludes that strong alkalies do not enter the cell until the surface is destroyed. The process is irreversible. He also states that the strong alkalies kill by affecting the plasma membrane and likewise on pages 534 and 547 he states that ammonia must affect the membrane since it produces changes in behavior similar to those produced by NaOH—vesicle formation, cessation of movement and finally death—but the

changes produced bear no relation to the speed of entrance. The results of the present experiments seem to confirm Harvey's conclusions that ammonia and certain ammonium salts exert their lethal effects on the plasma membrane and not on the internal protoplasm. The question may be raised that the non-toxicity of the injected salts is due to their outward diffusion from the cell. This is not probable for there is no reason to believe that the salts would diffuse out of the cell any faster than they would enter the cell. It may also be thought that if the toxicity of the ammonium salts is due to their actual passage—dissociated or undissociated—through the membrane or to a chemical combination between the salts and plasma membrane that the outward diffusion, if it occurs, from the injected cell would produce death. This, however, is not the case. So it may possibly be that the two sides of the membrane are different chemically or that the ammonium salts are adsorbed as molecules or ions on the external surface of the cell or they unite with some constituent of the outer surface of plasma membrane.

Summary.

A study was made by immersion and injection on the effects of the following ammonium salts: hydroxide, carbonate, chloride, phosphate, acetate and citrate on the protoplasm of *Amœba proteus* (?).

The effects of the ammonium salts are essentially due to the cations but may be modified by the anions.

The ammonium salts produce an increase in viscosity of the protoplasm in immersed amœbæ which is followed by a slight swelling of the protoplasm and disintegration of the cell. Injections of the salts, except the carbonate, into amœbæ produce a reversible increase in viscosity. The animals recover from dosages in amounts equal to one fourth the volume of the cell. Injection of the carbonate results in a disintegration of the cell.

When amœbæ are immersed in N/100 solutions of the salts and the cell membrane torn, a new membrane is formed over the injured surface.

These results seem to indicate that the toxicity of certain ammonium salts is due to their action on the plasma membrane and not on the internal protoplasm.

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